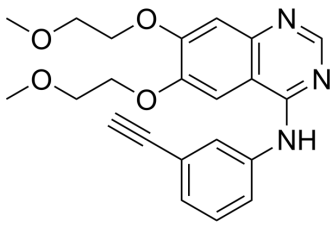
Erlotinib

Cat. No.:	HY-50896
CAS No.:	183321-74-6
Molecular Formula:	$C_{22}H_{23}N_3O_4$
Molecular Weight:	393.44
Target:	EGFR; Autophagy
Pathway:	JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Autophagy
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 1 year -20°C 6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : 3.94 mg/mL (10.01 mM; ultrasonic and warming and heat to 40°C)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5417 mL	12.7084 mL	25.4168 mL
	5 mM	0.5083 mL	2.5417 mL	5.0834 mL
	10 mM	0.2542 mL	1.2708 mL	2.5417 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 0.5% CMC-Na/saline water
   Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 50% PEG300 >> 50% saline
   Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Erlotinib (CP-358774) is a directly acting EGFR tyrosine kinase inhibitor, with an IC_{50} of 2 nM for human EGFR. Erlotinib reduces EGFR autophosphorylation in intact tumor cells with an IC_{50} of 20 nM. Erlotinib is used for the treatment of non-small cell lung cancer[1]. Erlotinib is a click chemistry reagent, itcontains an Alkyne group and can undergo copper-catalyzed azide-alkyne cycloaddition (CuAAc) with molecules containing Azide groups.

IC_{50} & Target

EGFR
2 nM (IC_{50}, Cell Free Assay)

In Vitro

Erlotinib (CP-358774) is also a potent inhibitor of the recombinant intracellular (kinase) domain of the EGFR, with an IC_{50} of 1 nM. The proliferation of DiFi cells is strongly inhibited by Erlotinib with an IC_{50} of 100 nM for an 8-day proliferation assay[1].

	The combination of B-DIM and Erlotinib (2 μM) results in a significant inhibition of colony formation in BxPC-3 cells when compared with either agent alone. The combination of B-DIM and Erlotinib (2 μM) results in a significant induction of apoptosis only in BxPC-3 cells when compare with the apoptotic effect of either agent alone[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Under the experimental conditions, the combination of B-DIM and Erlotinib (50 mg/kg, i.p.) treatment shows significant decrease (P <0.01) in tumor weight compared with untreated control[2]. Erlotinib (20 mg/kg, p.o.) significantly attenuates Cisplatin (CP)-induced body weight (BW) loss when compared to the CP+vehicle (V) rats (P<0.05). Erlotinib treatment significantly improves renal function in CP-N(normal control group, NC) rats. The CP+Erlotinib (E) rats show significant reduction of the levels of Serum creatinine (s-Cr) (P<0.05), blood urea nitrogen (BUN) (P<0.05), urinary N-acetyl-β-D-glucosaminidase (NAG) index (P<0.05), and significant increase of urine volume (UV) (P<0.05) and Cr clearance (Ccr) (P<0.05) compare to the CP+V rats[3]MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	The kinase reaction is performed in 50 μL of 50 mM HEPES (pH 7.3), containing 125 mM NaCl, 24 mM $MgCl_2$, 0.1 mM Na_3VO_4, 20 μM ATP, 1.6 μg/mL EGF, and 15 ng of EGFR, affinity purified from A431 cell membranes. The compound in DMSO is added to give a final DMSO concentration of 2.5%. Phosphorylation is initiated by addition of ATP and proceeded for 8 mm at room temperature, with constant shaking. The kinase reaction is terminated by aspiration of the reaction mixture and is washed 4 times with wash buffer. Phosphorylated PGT is measured by 25 mim of incubation with 50 μL per well HRP-conjugated PY54 antiphosphotyrosine antibody, diluted to 0.2 μg/mL in blocking buffer (3% BSA and 0.05% Tween 20 in PBS). Antibody is removed by aspiration, and the plate is washed 4 times with wash buffer. The colonmetric signal is developed by addition of TMB Microwell Peroxidase Substrate, 50 μL per well, and stopped by the addition of 0.09 M sulfuric acid, 50μL per well. Phosphotyrosine is estimated by measurement of absorbance at 450 nm. The signal for controls is typically 0.6-1.2 absorbance units, with essentially no back ground in wells without AlP, EGFR, or POT and is proportional to the time of incubation for 10 mm[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [2]	To test the viability of cells treated with B-DIM, Erlotinib, or the combination, BxPC-3 and MIAPaCa cells are plated (3,000-5,000 per well) in a 96-well plate and incubated overnight at 37°C. A range of concentrations for both B-DIM (10-50 μM) and Erlotinib (1-5 μM) is initially tested. Based on the initial results, the concentration of B-DIM (20 μM) and Erlotinib (2 μM) are chosen for all assays. The effects of B-DIM (20 μM), Erlotinib (2 μM), and the combination on BxPC-3 and MIAPaCa cells are determined by the standard MTT assay after 72 h and is repeated three times. The color intensity is measured by a Tecan microplate fluorometer at 595 nm. DMSO-treated cells are considered to be the untreated control and assigned a value of 100%. In addition to the above assay, we have also done clonogenic assay for assessing the effects of treatment[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2][3]	Mice[2] Female ICR-SCID (6-7 weeks old) mice are randomized into the following treatment groups (n=7): (a) untreated control; (b) only B-DIM (50 mg/kg body weight), intragastric once every day; (c) Erlotinib (50 mg/kg body weight), everyday i.p. for 15 days; and (d) B-DIM and Erlotinib, following schedule as for individual treatments. All mice are killed on day 3 following last dose of treatment, and their body weight is determined. One part of the tissue is rapidly frozen in liquid nitrogen and stored at −70°C for future use and the other part is fixed in formalin and processed for paraffin block. H&E staining of fixed tissue section is used to confirm the presence of tumor(s) in each pancreas. Rats[3] Six-week-old male Sprague-Dawley (SD) rats weighing 180 to 210 g are used. Cisplatin (CP) is freshly prepared in saline at a concentration of 1 mg/mL and then injected intraperitoneally in SD rats (n=28) at a dose of 7 mg/kg on day 0. To investigate the effect of Erlotinib, 28 CP-N rats are divided into two groups. Separate groups (n=14) each of animals are administered with either Erlotinib (20 mg/kg) (CP+E, n=14) or vehicle (CP+V, n=14) daily by oral gavage from day -1 (24 hours prior to the CP injection) to day 3. Vehicle-treated groups receive an equivalent volume of saline. Five male SD rats at the age of 6 weeks are used as a normal control group (NC, n=5). The NC rats are given an equivalent volume of saline daily by oral gavage from day -1 to day 3. At day 4 (96 hours after CP injection), each rat is anesthetized and sacrificed by exsanguination after the

cardiac puncture; blood is collected by cardiac puncture and kidneys are collected. Renal tissue is divided; separate portions are snap-frozen in liquid nitrogen or fixed in 2% paraformaldehyde/phosphate-buffered saline (PBS) for later use. All surgery is performed under diethyl ether gas anesthesia, and all efforts are made to minimize suffering.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Res. 2020 Oct;30(10):833-853.
- Nat Immunol. 2022 Feb;23(2):251-261.
- Bioact Mater. 2021 Nov 4;13:312-323.
- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Nat Commun. 2023 Apr 24;14(1):2342.

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REFERENCES

[1]. Moyer JD, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res. 1997, 57(21), 4838-4848.

[2]. Ali S, et al. Apoptosis-inducing effect of erlotinib is potentiated by 3,3'-diindolylmethane in vitro and in vivo using an orthotopic model of pancreatic cancer. Mol Cancer Ther, 2008, 7(6), 1708-1719.

[3]. Wada Y, et al. Epidermal growth factor receptor inhibition with erlotinib partially prevents cisplatin-induced nephrotoxicity in rats. PLoS One. 2014 Nov 12;9(11):e111728.

Caution: Product has not been fully validated for medical applications. For research use only.

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